The pH Tolerance of Embryos and Larvae of the Coot Clam, *Mulinia lateralis* (Say)[1,2]

BY

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(2 Text figures)

INTRODUCTION

Estuaries are a major habitat of bivalve mollusks and are one of the most complex environments found in nature. The coot clam, *Mulinia lateralis* (Say, 1822), is an ecologically important member of the estuarine fauna because it serves as food for many inshore species of animals, such as the scup, *Stenotomus chrysops* (Linnaeus), and other fishes (Verrill, 1873); the starfish, *Asterias forbesi* (Desor, 1848), and the oyster drills, *Eupleura caudata* and *Urosalpinx cinerea* (Say, 1822) (C. L. MacKenzie, Jr., personal communication); and the greater scaup duck, *Aythya marila,* and the lesser scaup duck, *Aythya affinis* (Cronan, 1957). Of the many interacting factors – biological, chemical, and physical – that affect bivalves in these waters, pH has received less attention than any other major variable.

In laboratory experiments Loosanoff & Tommers (1947) found that adult American oysters, *Crassostrea virginica* (Gmelin, 1791), kept in sea water at pH 4.25 remained open, on an average, 76% of the time, but pumped only 10% as much water as did the controls at pH 7.75. The pumping rate of oysters kept at pH 6.75 and 7.00 initially was greater than that of the control animals, but later decreased to less than that of the controls. Prytherch (1928), who took pH measurements at several stations in Milford Harbor and the Milford area of Long Island Sound, found that it ranged from 7.2 - 8.4 during the day and suggested that oysters, *C. virginica,* spawned at pH 7.8 - 8.2 in these waters. On this basis he concluded that low pH inhibited oyster spawning and that oysters in Milford Harbor spawned at high tide because this was the only tidal stage at which the pH was between 7.8 and 8.2. Calabrese & Davis (1969) found that the minimum and maximum pH levels at which American oysters were capable of spawning were 6.0 and 10.0, but that eggs and sperm released at pH 6.0 and 10.0 lost their viability within 2 - 4 hours, due to a combination of pH effects and aging.

Korringa (1941), quoting Gaarder (1932) and Gaarder & Spärck (1932), stated that larvae of the European oyster, *Ostrea edulis* (Linnaeus, 1758), died when the pH level in their oyster polls exceeded 9.0. Calabrese & Davis (1966), who studied the effect of pH on embryonic development and survival and growth of larvae of *Crassostrea virginica* and the hard clam, *Mercenaria mercenaria* (Linnaeus, 1758), found that some oyster larvae survived throughout a pH range from 6.00 - 9.00 but that satisfactory growth was limited to a pH range from 6.75 to 8.75. Some clam larvae survived between pH levels of 6.25 - 9.00, but growth of these larvae was satisfactory only within the pH range from 6.75 to 8.50.

Although the pH of oceanic waters ranges from 7.5 to 8.5, the pH levels in bays and estuaries may decrease to 7.0 or lower, due to dilution and production of H_2S (Sverdrup *et al.*, 1942). Such bodies of water constitute a major habitat of bivalves, and Davis & Calabrese (1964) suggested that these regions may be exceedingly important also as nursery grounds for the larval stages. Since bivalve larvae must encounter, at times, a wide range of pH in their natural habitat, it seems possible that success or failure of recruitment in some areas may be determined by such exposure.

The present study was designed to determine the pH tolerance of the embryonic and larval stages of coot clams under experimental conditions.

[1] Part of a dissertation submitted to the Graduate Faculty of the University of Connecticut in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Contribution No. 60 from the Marine Research Laboratory, University of Connecticut.

METHODS

Methods for maintaining oysters, *Crassostrea virginica*, in spawning condition and obtaining fertilized eggs throughout the year and for spawning other species of bivalves and rearing their larvae were described by Loosanoff & Davis (1963). To determine the effect of pH on embryonic development of *Mulinia lateralis*, 12 000 to 15 000 fertilized eggs were placed into each of a series of 1-liter polypropylene beakers containing filtered, ultraviolet-treated sea water (salinity $27 \pm 0.5‰$) and test conditions were established. In 3 experiments fertilized eggs were placed in the beakers and the pH of duplicate cultures was adjusted with HCl or NaOH to levels ranging from 5.75 to 9.25 at 0.25-unit intervals. One pair of cultures maintained at the pH of the laboratory sea water (7.30 - 7.80) served as controls. All cultures were kept at ambient room temperature ($21 \pm 1°C$). To determine the effect of pH on embryonic development, the larvae from each culture were collected on a stainless steel screen of mesh size small enough to retain them, after 48 hours at the experimental conditions. The larvae were resuspended in a 250-ml graduated cylinder and, after thorough mixing to assure random distribution of the larvae, a 4-ml quantitative sample was removed and preserved in 5% neutral formalin. The samples were examined under a compound microscope and the number of normal larvae that had developed was counted. The percentage of embryos developing to normal straight-hinge larvae at each pH level was calculated as a percentage of the maximum number developing normally in any pair of cultures in that experiment. The percentages reported in this study are the averages of the results in the 3 experiments.

Three additional experiments were run to determine the effect of various pH levels on the survival and growth of larvae. In each experiment a known number of larvae (usually 10 000 to 14 000), which had been reared to the 48-hour straight-hinge stage under normal conditions, were placed into each of the series of 1-liter beakers and the different pH levels established as above. The sea water in all cultures was changed 3 times a week to eliminate metabolic waste products and experimental conditions were reestablished. Supplemental algal food, consisting of *Isochrysis galbana*, *Monochrysis lutheri*, and *Chlorella* sp. (580) (Indiana University Collection No. 580), was added to each beaker daily, following the procedures of Davis & Guillard (1958). When food was added on days that the sea water was not changed, the pH was readjusted following the addition of food. In all larval experiments 50 mg/l of Sulmet were added to all cultures to prevent possible disease-induced mortality which might obscure the effects of the factor being tested. (Sulmet, sodium sulfamethazine, is a trade name of American Cyanamid Co. Mention of trade names does not imply endorsement of the product by the Bureau of Commercial Fisheries.) In all experiments the cultures were kept in a constant-temperature bath at $25 \pm 1°$ C. Experiments were terminated when larvae in the fastest growing cultures reached setting size (6 - 8 days). Quantitative samples were taken from each culture at the termination of an experiment in a similar manner as for experiments with embryos. The number of larvae that had survived the experimental treatment was counted and 100 (if available) were measured to the nearest 5μ with an ocular micrometer in a compound microscope. The number of larvae surviving and the increase in mean length were calculated as a percentage of the maximum number surviving and maximum increase in mean length, respectively, in any one pair of cultures within that experiment. The method for determining the number of larvae surviving or the percentage of bivalve embryos developing into normal straight-hinge larvae is considered accurate to approximately ±10% (Davis, 1958).

Since prior experience suggested that buffers were not adequate for maintaining desired pH levels for long periods of time (Calabrese & Davis, 1966), it was necessary to readjust the pH at approximately 12-hour intervals. The pH was measured with a line-operated, solid-state pH meter having a readability of 0.02 pH unit and a repeatability of 0.01 pH unit. The range and average pH for each initial pH established are shown in Figure 1.

EFFECT OF pH ON EMBRYONIC DEVELOPMENT

The percentage of *Mulinia lateralis* embryos developing normally, within the pH range from 7.25 to 8.25, did not vary significantly (Table 1 and Figure 2), but pH 7.75 appeared to be the optimum for such development. The percentage of fertilized eggs that developed normally was reduced to 59.5 at pH 7.00 and 61.8 at pH 8.50. Embryonic development decreased precipitously below pH 7.00 and above pH 8.50; only 26.5% and 5.1% of the embryos developed normally at pH 6.75 and 8.75, respectively. At pH 6.25 and pH 9.00 few or no embryos developed into shelled larvae.

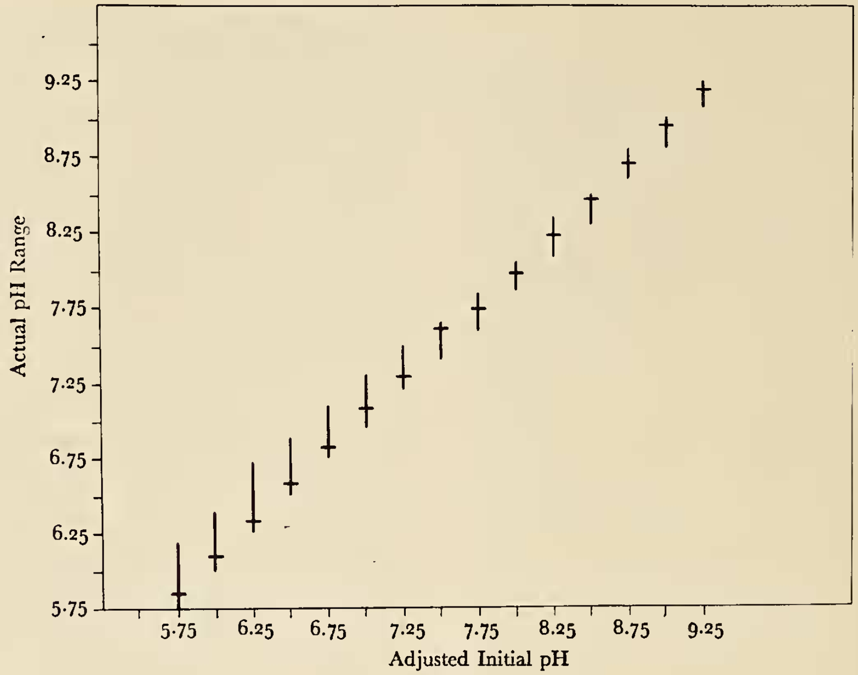

Figure 1

Maximum range of pH (vertical bar) and average pH (horizontal bar) for each initial pH. The "adjusted initial pH" was established at the beginning of each experiment and readjusted to this level at 12-hour intervals, by the addition of HCl or NaOH

EFFECT OF pH ON SURVIVAL

Survival of larvae increased sharply from 14.2% at pH 6.00 to 65.1% at pH 6.25, was about normal (70% or more of maximum) throughout the range from pH 6.50 to 8.75, and decreased sharply from 83.8% at pH 8.75 to 20.2% at pH 9.00 (Table 1 and Figure 2). No larvae survived at pH 9.25. Thus, the larvae were able to survive throughout a much wider pH range than the embryos.

EFFECT OF pH ON GROWTH

The pH range for satisfactory growth (70% or more of maximum) was 7.00 to 8.50 (Table 1 and Figure 2). Therefore, the range for normal growth was slightly narrower than the pH range of 6.50 to 8.75 for satisfactory survival. The maximum increase in mean length occurred at pH 7.25. The percentage increase in mean length varied only slightly within the pH range from 7.00 to 8.50, but below pH 7.00 and above 8.50 the rate of growth decreased rapidly.

IMPLICATION FOR DISTRIBUTION AND SURVIVAL IN NATURE

Embryonic development of *Mulinia lateralis* occurred within the pH range from 6.25 to 8.75, and some larvae survived from 5.75 to 9.00. The pH range for a satisfactory percentage of embryos to develop, however, was 7.25 to 8.25 and for survival of larvae it was 6.50 to 8.75. Even though a substantial percentage (65.1) of larvae was able to survive at pH 6.25, growth was negligible and good growth was not attained until pH 6.75 was reached. A significant percentage (83.8) of larvae survived at pH 8.75 and showed fair growth (50.4%). CALABRESE & DAVIS (1966) reported that *Mercenaria mercenaria* and *Crassostrea virginica* embryos developed normally within

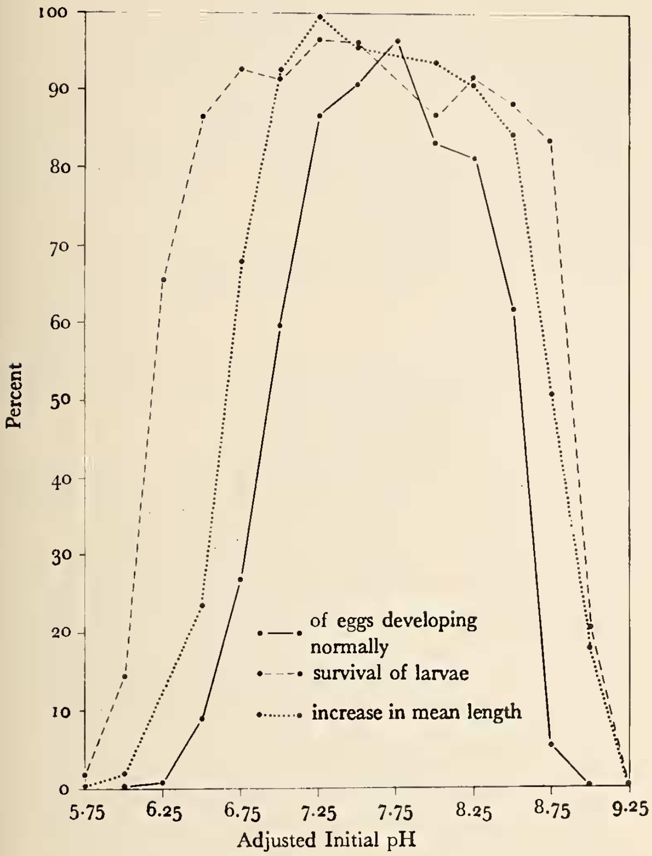

Figure 2

The pH tolerance of embryos and larvae of *Mulinia lateralis*, as indicated by percentage of embryos that developed normally, percentage of larvae that survived, and percentage increase in mean length of larvae

the pH range 7.00 - 8.75 and 6.75 - 8.75, respectively; thus, *Mulinia lateralis* embryos appear to be less tolerant to pH variations than either of these 2 species. *Mulinia lateralis* larvae attain maximum growth at a pH level lower than that reported for larvae of either *Mercenaria mercenaria* (pH 7.50 - 8.00) or *C. virginica* (pH 8.25 - 8.50), as reported by Calabrese & Davis (*op. cit.*). It can be concluded, therefore, that for successful recruitment of *Mulinia lateralis* the pH of the waters that form their principal habitat is limited primarily by the pH range for embryonic development; this range must not fall below 7.25 nor rise above 8.25.

Table 1

Percentage Development of *Mulinia lateralis* Embryos, Larval Survival, and Increase in Mean Length of Larvae at Various pH Levels[3]

pH	Percentage development of embryos	Percentage survival of larvae	Percentage increase in mean length of larvae
5.75	0.0	1.8	0.0
6.00	0.0	14.2	1.7
6.25	0.4	65.1	3.8
6.50	9.4	86.4	23.8
6.75	26.5	92.8	67.7
7.00	59.5	91.3	92.7
7.25	86.9	96.6	99.9
7.50	90.9	96.0	95.8
7.75	95.7	91.4	94.5
8.00	83.1	86.7	93.9
8.25	81.3	91.6	90.7
8.50	61.8	88.3	84.2
8.75	5.1	83.8	50.4
9.00	0.0	20.2	17.2
9.25	0.0	0.0	0.0

[3] Development, survival, and increase in mean length are expressed as percentage of the greatest development, survival, and increase in mean length at any pH within that experiment. Figures are averages for duplicate cultures at each pH level in all 3 experiments.

Laboratory experiments have shown that high concentrations of silt can lower the pH of sea water to 6.40 (Calabrese & Davis, 1966), or below the lower limit for normal development of embryos of the coot clam. It is apparent, therefore, that heavy silting, or any pollution that can change the pH of sea water, could cause failure of recruitment of these clams.

SUMMARY

Some embryos of *Mulinia lateralis* developed into normal larvae from pH 6.25 to 8.75, but development was satisfactory (70% or more of maximum) only within the range from 7.25 to 8.25, and was highest at 7.75. Some larvae survived from pH 5.75 to 9.00, but survival was satisfactory (70% or more of maximum) only within the

range from 6.50 to 8.75, and was highest at 7.25 to 7.50. Larvae grew satisfactorily within the pH range from 7.00 to 8.50; pH 7.25 was the optimum for growth.

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